

What is claimed is:

1. A method for the electrochemical detection of an analyte molecule by means of a detection electrode, the method comprising:

- 5 (a) immobilizing capture molecules, which are capable of binding the analyte molecule to be detected, on the detection electrode;
- (b) contacting the electrode with a solution supposed to contain the analyte molecule to be detected;
- 10 (c) allowing the analyte molecule contained in said solution to bind to the capture molecules on the electrode, thereby allowing formation of complexes between a capture molecule and an analyte molecule, said complexes forming a first layer on the detection electrode;
- (d) contacting the detection electrode with an electrochemical activator, wherein said electrochemical activator has a electrostatic net charge
- 15 that is complementary to the electrostatic net charge of the complex formed between a capture molecule and an analyte molecule, thereby forming a second layer on the electrode, wherein the second layer and the first layer together form a conducting bilayer;
- (e) contacting the detection electrode with an agent capable of
- 20 transferring electrons to or from the electrochemical activator from or to the electrode, respectively;
- (f) performing an electrical measurement at the detection electrode, and;
- (g) detecting the analytes by comparing the result of the electrical
- 25 measurement obtained with that of a control measurement.

2. The method of claim 1, wherein the electrochemical activator is a polymeric redox mediator capable of transferring electrons between the analyte and the electrode.

3. The method of claim 2, wherein the electrochemical activator comprises metal ions.

4. The method of claim 3, wherein the metal ions are selected from the group consisting of silver, gold, copper, nickel, iron, cobalt, osmium, ruthenium, and mixtures thereof.
- 5 5. The method of claim 4, wherein the electrochemical activator is selected from the group consisting of poly(vinyl ferrocene), poly(vinyl ferrocene)-co-acrylamide, poly(vinyl ferrocene)-co-acrylic acid, and poly(vinyl ferrocene)-co-acrylamido-(CH₂)_n-sulfonic acid, and poly(vinyl ferrocene)-co-acrylamido-(CH₂)_n-phosphonic acid, wherein n = 0-12.
- 10 6. The method of claim 1, wherein the agent capable of transferring electrons to or from the electrochemical activator is an enzyme or an enzyme-conjugate.
- 15 7. The method of claim 6, wherein the enzyme is an oxidoreductase or a mixture of oxidoreductases.
- 20 8. The method of claim 7, wherein the oxidoreductase is selected from the group consisting of glucose oxidase, hydrogen peroxidase, lactate oxidase, alcohol dehydrogenase, hydroxybutyrate dehydrogenase, lactic dehydrogenase, glycerol dehydrogenase, sorbitol dehydrogenase, glucose dehydrogenase, malate dehydrogenase, galactose dehydrogenase, malate oxidase, galactose oxidase, xanthine dehydrogenase, alcohol oxidase, choline oxidase, xanthine oxidase, choline dehydrogenase, pyruvate dehydrogenase, pyruvate oxidase, oxalate oxidase, bilirubin oxidase, glutamate dehydrogenase, glutamate oxidase, amine oxidase, NADPH oxidase, urate oxidase, cytochrome C oxidase, and actechol oxidase.
- 25 9. The method of claim 1, wherein the capture molecules are capable of specifically binding the analytes to be detected.
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10. The method of claim 1, wherein the analyte to be detected is selected from the group consisting of nucleic acids, oligonucleotides, proteins, peptides, oligosaccharides, polysaccharides and complexes thereof.
- 5 11. The method of claim 10, wherein the analyte to be detected is a nucleic acid molecule.
12. The method of claim 11, wherein the nucleic acid molecule has a pre-defined sequence.
- 10 13. The method of claim 12, wherein the nucleic acid molecule comprise at least one single-stranded region.
- 15 14. The method of claim 13, wherein the capture molecule is at least one nucleic acid probe having a sequence complementary to a single-stranded region of the nucleic acid molecule to be detected.
- 20 15. The method of claim 10, wherein the analyte to be detected is a protein or a peptide.
- 25 16. The method of claim 1, wherein a blocking agent is immobilized on the electrode prior to contacting the electrode with the solution supposed to contain the analyte molecule.
- 30 17. A method for the electrochemical detection of an analyte molecule by means of a detection electrode, the method comprising:
- (a) immobilizing capture molecules, which are capable of binding the analyte molecule to be detected, on the detection electrode;
 - (b) contacting the electrode with a solution supposed to contain the analyte molecule to be detected;

(c) allowing the analyte molecule contained in said solution to bind to the capture molecules on the electrode, thereby allowing formation of complexes between a capture molecule and an analyte molecule, said complexes forming a first layer on the detection electrode;

5 (d) contacting the detection electrode with an electrochemical activator, wherein said electrochemical activator has an electrostatic net charge that is complementary to the electrostatic net charge of the complex formed between a capture molecule and an analyte molecule, thereby forming a second layer on the electrode, wherein
10 the second layer and the first layer together form a conducting bilayer, and wherein the capture molecules are capable of transferring electrons to or from the electrochemical activator from or to the electrode, respectively;

15 (e) performing an electrical measurement at the detection electrode, and;

(f) detecting the analytes by comparing the result of the electrical measurement obtained with that of a control measurement.

20 18. An electrode arrangement, comprising a detection electrode, suitable for carrying out an electrochemical detection of an analyte molecule as defined in claim 1, comprising:

(a) a first layer on the detection electrode comprising complexes between a capture molecule, which is capable of binding the analyte molecule to be detected, and an analyte molecule; and

25 (b) a second layer comprising an electrochemical activator, wherein said electrochemical activator has an electrostatic net charge that is complementary to the electrostatic net charge of the complex formed between a capture molecule and an analyte molecule, wherein the second layer and the first layer together form a conducting bilayer.

30 19. The electrode arrangement of claim 18, wherein the electrochemical activator is a polymeric redox mediator capable of transferring electrons between the analyte and the electrode.

20. The electrode arrangement of claim 19, wherein the agent for increasing conductivity of the analytes contains metal ions.

5 21. The electrode arrangement of claim 20, wherein the metal ions are selected from the group consisting of silver, gold, copper, nickel, iron, cobalt, osmium, ruthenium and mixtures thereof.

10 22. The electrode arrangement of claim 18, further comprising an agent capable of transferring electrons to or from the polymeric redox mediator from or to the electrode, respectively, wherein the agent is bound to, intercalated in or associated with the conducting bilayer

15 23. The electrode arrangement of claim 22, wherein the agent is an enzyme or an enzyme-conjugate.

24. Use of an electrode arrangement of claim 18 as biosensor.

20 25. A biosensor for the electrochemical detection of an analyte molecule, comprising:

- (a) an detection electrode;
- (b) a first layer on the detection electrode comprising complexes between a capture molecule, which is capable of binding the analyte molecule to be detected, and an analyte molecule; and
- 25 (c) a second layer comprising an electrochemical activator, wherein said electrochemical activator has an electrostatic net charge that is complementary to the electrostatic net charge of the complex formed between a capture molecule and an analyte molecule, wherein the second layer and the first layer together form a conducting bilayer.

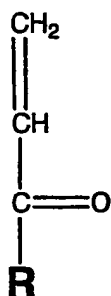
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26. A water soluble redox polymer, comprising:

- (a) a first monomer unit comprising a polymerizable ferrocene derivative;
- and

- (b) a second monomer unit comprising an acrylic acid derivative having a primary acid or base functional group capable of acquiring a net charge.

- 5 27. The redox polymer of claim 26, wherein the second monomer unit comprises an acrylic acid derivative having an terminal primary acid or base functional group capable of acquiring a net charge.
- 10 28. The redox polymer of claim 26, wherein the acrylic acid derivative is represented by the general formula (I)



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- wherein R is selected from the group consisting of $\text{C}_n\text{H}_{2n}\text{-NH}_2$, $\text{C}_n\text{H}_{2n}\text{-COOH}$, $\text{NH-C}_n\text{H}_{2n}\text{-PO}_3\text{H}$, and $\text{NH-C}_n\text{H}_{2n}\text{-SO}_3\text{H}$, wherein the alkyl chain can be optionally substituted, and wherein n is an integer from 0 to 12.
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29. The redox polymer of claim 26, wherein the polymerizable ferrocene derivative is selected from the group consisting of vinyl-ferrocene, acetylene-ferrocene, styrene-ferrocene and ethylene oxide-ferrocene.
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30. The redox polymer of claim 29, wherein the ferrocene derivative is vinyl ferrocene.

31. The redox polymer of claim 26, wherein the molecular weight of the redox polymer is between about 1000 and 5000 Daltons.
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32. The redox polymer of claim 26, wherein the ferrocene loading in the redox polymer is between about 3% and 14%.

33. A process for preparing a water soluble, redox polymer, said process comprising:

polymerising a first monomer unit comprising a polymerizable ferrocene derivative with a second monomer unit comprising an acrylic acid derivative having an acid or base functional group capable of acquiring a net charge, wherein said polymerization is carried out in an aqueous alcoholic medium.

34. The process of claim 33, wherein the aqueous alcoholic medium comprises ethanol and water in a volumetric ratio of between 2:1 and 3:1.

35. The process of claim 33, wherein the polymerization is initiated by adding a free radical initiator.

36. The process of claim 35, wherein the free radical initiator is selected from the group consisting of ammonium persulfate, potassium persulphate and sodium persulfate.

37. The process of claim 35 wherein the weight ratio of free radical initiator added is between about 20 mg to 40 mg per 1 gram of monomer.

38. The process of claim 33, wherein polymerization is carried out under reflux at a temperature of between about 60 °C to 80 °C.

39. The process of claim 33, wherein polymerization is carried out in an inert gas atmosphere.

40. The process of claim 33, wherein polymerization is carried out for about 24 hours.

41. The process according to claim 33, further comprising: forming a pre-reaction mixture prior to polymerizing said first and second monomers, comprising:

dissolving the acrylic acid derivative monomer unit in an aqueous alcoholic medium, then

adding the free radical initiator, and then

adding the polymerisable ferrocene derivative monomer unit to form the pre-reaction mixture.

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42. The process of claim 41, wherein the feeding ratio of acrylic acid derivative to polymerizable ferrocene derivative in the pre-reaction mixture is between about 5% and 15% of the weight of monomer added.

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43. The process of claim 41, wherein the polymerizable ferrocene derivative monomer unit is dissolved in an aqueous alcoholic medium prior to being added.

- 15 44. The process of claim 41, further comprising precipitating the redox mediator in an organic solvent.

45. The process according to Claim 40, wherein the organic solvent is selected from the group consisting of an ether and ketone.

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